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ARTICLE

Considerations for Consistently Applying Flow-Through Chloramine-T Treatments to Hatchery Raceways

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Abstract

Chloramine-T (CLT) was recently approved for use in the United States by the U.S. Food and Drug Administration (FDA) to control mortality in selected freshwater-reared finfishes diagnosed with bacterial gill disease or external columnaris disease. In support of this approval, we conducted a study to determine if a target dose of 12 mg/L CLT could be delivered for 60 min via a "charged," flow-through treatment protocol. The study was conducted in two production-size, linear-design, plug flow raceways devoid of fish. Each raceway was dosed twice, resulting in four replicate trials ($N = 4$). During each trial, CLT was added under static conditions to establish a target concentration of 12 mg/L. Inflow water was then resumed, and additional CLT stock solution was metered into the raceway for the 60-min treatment period. Water samples were collected from a matrix of 27 sampling locations (3 positions along raceway length × 3 positions across raceway width × 3 depths) for colorimetric determination of CLT concentrations at 0 min (after charging but before resuming water inflow), 30 min, and 60 min. Chloramine-T doses delivered (data from all sampling locations and times pooled) did not vary from trial to trial. Median CLT doses delivered were almost always less than 12 mg/L; however, all had corresponding 95% confidence intervals within 9–15 mg/L. Overall, the results of our study demonstrated that the treatment method can be used to deliver a target dose of CLT for 60 min in production-size raceways in a manner that was found acceptable to the FDA.

Chloramine-T (CLT; *N*-sodium-*N*-chloro-*p*-toluene sulfonamide; $C_7H_7ClNNaO_2S{\cdot}^3H_2O$) is a biocide used worldwide as a disinfectant and antiseptic. The chemical is a white, crystalline powder with a weak chlorine odor and is a pure compound with no inactive ingredients. Hypochlorite ions that form when CLT is dissolved in water (Booth and McDonald 1988) are strong oxidizers that can quickly destroy cellular material and disrupt essential cell processes (Powell and Clark 2003). Chloramine-T has been shown to effectively control the mortality associated with bacterial gill disease (BGD) in salmonids

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(from 1980; Speare and Ferguson 1989; Bullock et al. 1991; Thorburn and Moccia 1993; Ostland et al. 1995; Bowker and Erdahl 1998; Bowker et al. 2008b) and with external columnaris in Largemouth Bass *Micropterus salmoides* and Bluegill *Lepomis macrochirus* (Bowker et al. 2013). The toxicity of CLT to fishes has also been investigated (Bills et al. 1988; Powell et al. 1995, 1998; Powell and Perry 1996, 1998; Sanchez et al. 1996, 1997; King and Farrell 2002; Powell and Harris 2004; Gaikowski et al. 2008, 2009; Bowker et al. 2011), with results indicating an adequate margin of safety when it is used according to established treatment protocols. These data were used, in part, to support the May 2014 approval of CLT by the U.S. Food and Drug Administration (FDA) for use to control mortality in freshwater-reared salmonids diagnosed with BGD and in Walleye *Sander vitreus* and warmwater finfish diagnosed with external columnaris.

Maintaining healthy rearing conditions, providing proper nutrition, and routinely monitoring fish health can help minimize disease outbreaks. The timely administration of therapeutic drugs according to proven treatment regimens (dose, frequency, and duration) can help to minimize mortality when disease outbreaks occur. It is easy to ensure that treatments are administered at appropriate frequencies, durations, and doses in static baths. However, it can be difficult to ensure that effective doses are achieved and maintained throughout the treatment period when using waterborne chemicals like CLT in flow-through systems. For example, Rach et al. (1997) and Rach and Ramsay (2000) found that target doses of hydrogen peroxide (HP) were not always delivered in flow-through egg incubators or production-size raceways. On a small scale, Saez and Bowser (2001) showed that a target dose of HP could be delivered for 60 min in a flow-through system but only when the water contents of the tank was first "spiked" or "charged" to the desired dose as a standing bath.

As part of the effort to obtain FDA approval of the use of CLT in U.S. aquaculture, researchers at the U.S. Fish and Wildlife Service (FWS) Aquatic Animal Drug Approval Partnership program conducted CLT efficacy (EFF) studies on Rainbow Trout *Oncorhynchus mykiss,* Apache Trout *O. apache*, and Chum Salmon *O. keta* (Bowker et al. 2008b), and target animal safety (TAS) studies on Rainbow Trout (Bowker et al. 2011). Though all CLT doses administered in these studies were analytically verified and found to be within FDA-acceptable limits (i.e., target dose $\pm$ 25%), all had been administered in standing-bath treatment conditions. No CLT EFF or TAS studies had been conducted to generate FDA-acceptable data under flow-through treatment conditions, and there was concern that this "data gap" would restrict the use of CLT to standing-bath treatments only. Such treatment protocols may not be feasible in large-scale, flow-through systems. Transferring fish between rearing tanks and treatment tanks is impractical, and the extra handling and associated stress would likely exacerbate the disease outbreak. Halting flow, even temporarily, during treatment can cause similar complications by stressing the fish via exposure to reduced water quality. In the present work, our objective was to determine whether a target dose of 12 mg CLT/L ($\pm$25%) could be delivered for 60 min with a charged, flow-through treatment protocol.

METHODS

This study was conducted at the FWS Bozeman Fish Technology Center (BFTC; Bozeman, Montana). Chloramine-T (CAS 127-65-1) was obtained from Akzo-Nobel Chemical (Amsterdam); this product was the same used in the EFF and TAS studies we conducted as described above. Several 5-g CLT samples were sent to the U.S. Geological Survey Upper Midwest Environmental Sciences Center (La Crosse, Wisconsin) for assay by high-performance liquid chromatography (HPLC), and all samples were found to be >99% pure.

Experimental setup and dosing procedures.—Two concrete, production-size, laminar-flow raceways (17.7 m long $\times$ 1.8 m wide $\times$ 1.1 m deep) were used in the study (Figure 1). Single-pass, flow-through spring water was delivered by gravity flow to the head of each raceway at a mean rate of 680 $\pm$ 38 L/min, resulting in >2.5 water exchanges/h. Effluent water drained from the tail of each raceway through a standpipe. Each raceway had an approximate water depth of 0.51 m (tail sloped to a depth of 0.64 m) and volume of 14.5 m^3. During the study, mean water temperature and dissolved oxygen concentration were 7.6°C ($\pm$0.1, SD) and 9.4 mg/L ($\pm$0.7), respectively. Based on historical records, the hardness, alkalinity, and pH of the spring water at the BFTC are approximately 180 mg/L (as $CaCO_3$), 170 mg/L (as $CaCO_3$), and 7.8, respectively.

Each raceway was dosed twice with CLT; thus, four independent trials were conducted from July 24–27, 2001. To simulate minimal mixing conditions (a worst-case scenario for homogenous treatment application), raceways were not stocked with fish. We chose 12 mg/L as the target dose because it is the minimum effective concentration approved by FDA. Before each trial, the amount of CLT needed to achieve the 12-mg/L target dose (i.e., charge the raceway) was calculated from equations in Piper et al. (1982), weighed out to the nearest 0.01 g, and dissolved in a container holding 34 L of spring water. A concentrated solution of CLT (in spring water) was prepared for metering into the raceway to maintain concentrations during the treatment period. The concentration was based on inflow rates and the volume of the water delivery system (chicken waterer plus glass siphon; Figure 2). Note that a larger volume of this solution (20–30% in excess of the needed volume) was prepared to ensure that the metering system did not run low (or empty completely) during treatment. By preparing a volume such that 20–30% remained in the system at the end of the treatment period, head pressure and constant flow were maintained during treatment.

Each trial began by charging a raceway to achieve the 12-mg/L target dose. A raceway was charged by turning off the inflowing water (thus creating a standing bath), pouring the

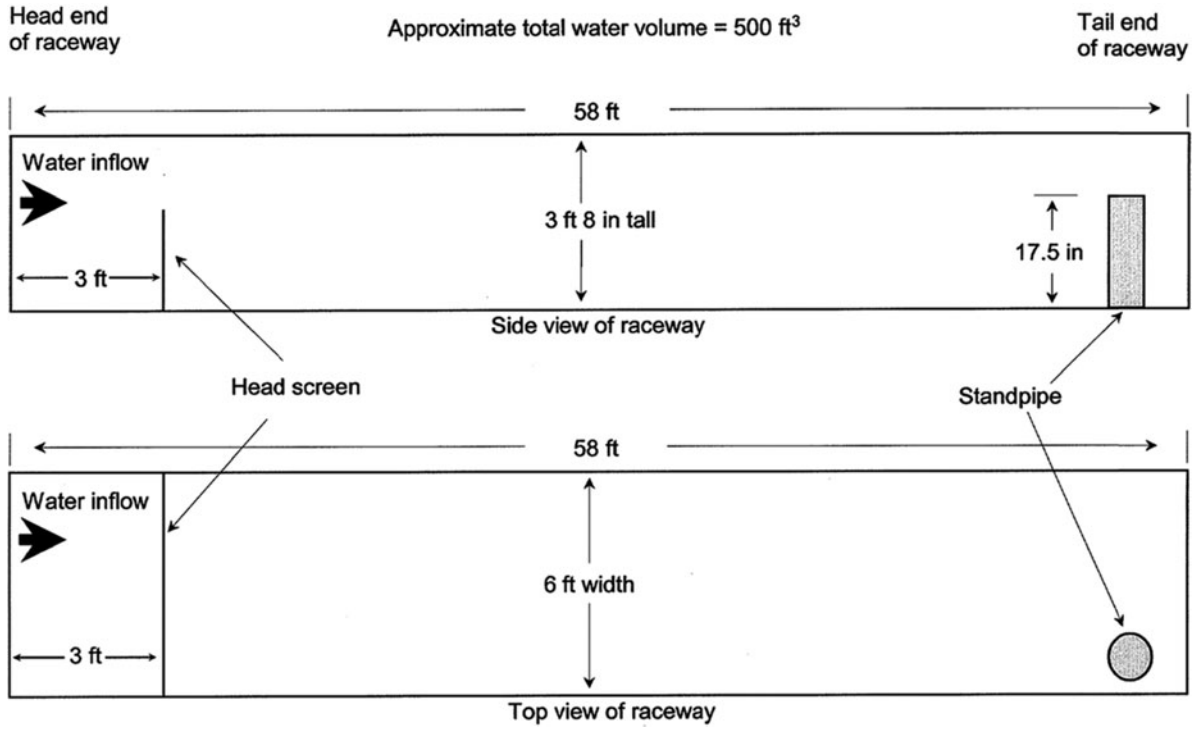

FIGURE 1. Schematic illustrating the raceways used to evaluate CLT application in a flow-through system.

34 L of CLT stock solution throughout the raceway, and manually mixing the stock solution into the raceway water with clean plastic leaf rakes. The inflowing water was then resumed, and the target dose was maintained for 60 min by metering CLT stock solution into the inflowing water with the chicken waterer delivery system. Position of valves on incoming water lines were premarked to facilitate reestablishing water flows.

Collection of chloramine-T data.—During each trial, water samples were collected from throughout the raceway and measured for CLT. Each raceway was divided into three sections (head, middle, and tail), and a sampling station was established within each section (Figure 3). Water was collected from a matrix of positions across the width and depths at each sampling station. Thus, water was collected from 27 fixed sampling locations in each raceway: near-right side, midline, and near-left side of the raceway; the near-surface, middepth, and near-bottom of the raceway; and head, middle, and tail of the raceway (Figure 3).

During each trial, water samples were collected for CLT dose verification and quality control at elapsed times of 0 min (immediately after charging the raceway), 30 min, and 60 min (immediately after stopping the flow from the chicken waterer); thus, 12 sampling events were conducted during the study. During each sampling event, 29 water samples (50–60 mL per sample) were collected in 0.9–2.5 min (mean collection time = 1.5 min) with a sampling apparatus designed and built for this purpose (Figure 4; Bowker et al. 2008a). Twenty-seven of the water samples collected during each sampling event were used for CLT dose verification, and two additional samples were collected from one sampling location and used for quality control to assess precision. Thus, a total of 324 samples were collected for CLT dose verification: 4 trials $\times$ 3 time points $\times$ 27 sampling locations).

Chloramine-T concentrations were measured to the nearest 0.1 mg/L with a HACH Chlorine Pocket Colorimeter (HACH Company, Loveland, Colorado; Dawson et al. 2003). Water samples collected and measured for CLT for quality control purposes ($n = 24$) indicated that the dose verification process had been done with reasonable precision and accuracy during all four trials. Inadvertently, two water samples collected 30 min into trial 2 were not tested with the colorimeter; thus, the final CLT dose verification database consisted of 322 measurements ($n = 81$, 79, 81, and 81 measurements for trials 1–4, respectively).

Data analysis.—We used a logistic regression approach to examine whether the probability of CLT concentration being maintained within $\pm$ 25% of the target concentration (i.e., target concentration = 12 mg/L, acceptable range = 9–15 mg/L) throughout the treatment period was influenced by sampling location within the raceway (length, width, and depth) across two trials per raceway. A first-order autoregressive covariance structure was used to account for repeated measurements conducted at each raceway sampling location within each trial. A stepwise model selection procedure was used to determine if the main effects of raceway, location nested within each raceway, and time as well as all possible interactions influenced the probability of maintaining the target CLT concentration; effects were entered

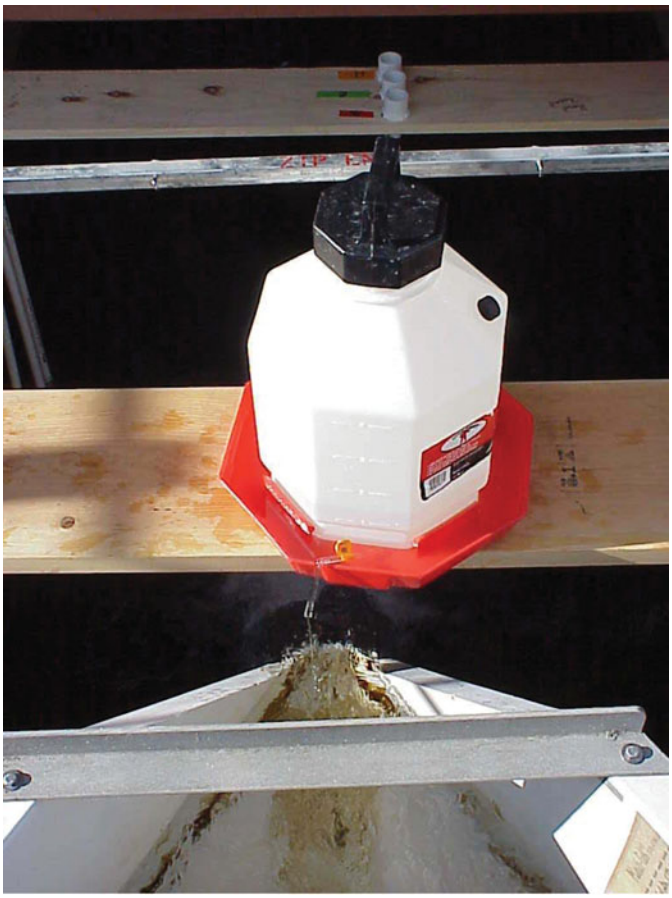

FIGURE 2. Photograph of the water delivery system composed of a chicken waterer plus a glass siphon.

into the model if significant at the alpha = 0.05 level and retained in the model if they maintained that significance level after the entry of other significant terms. The GENMOD procedure in SAS version 9.3 (SAS Institute, Cary, North Carolina) was used for statistical analyses.

RESULTS

The probability of maintaining target concentrations of CLT was different between raceways ($Z = 2.60$, $P = 0.009$), with raceway 1 maintaining target concentrations 90% of the time and raceway 2 maintaining target concentrations 97% of the time across all sampling locations within the raceways. All other effects were insignificant ($P < 0.05$) and were therefore not included in the model. Only two measurements exceeded the range of acceptable concentrations, both of which occurred in raceway 1 at the beginning of the treatment period (i.e., 0 min elapsed). Mean CLT concentrations were always within acceptable limits of the target concentration (i.e., 9–15 mg/L). There was a general decrease in mean concentration over time in both raceways, with the decrease being more pronounced in raceway 1 (Figure 5). Concentrations also tended to be more homogenous across raceway 2 than across raceway 1.

DISCUSSION

Our study demonstrated that a charged, flow-through treatment protocol would be an acceptable alternative when static baths are deemed excessively stressful to fish or not logistically possible. The methodology used was repeatable in that results were relatively consistent among trials, among sampling locations within raceways, and over time during the treatment periods. However, most CLT doses delivered were less than the target dose, and mean CLT doses delivered tended to decrease from 0 to 30–60 min (data from all raceway sections combined). These results suggest a number of factors that might influence the success of charged, flow-through protocols: specifically, accuracy in determining standing volumes, accuracy in measuring inflow and metering rates during the treatment period, and the degree of water mixing and homogeneity during treatment.

The aliquots of CLT used to charge a raceway to 12 mg/L and maintain that dose for 60 min were based on estimates of standing water volume and water inflow. If standing water volume was underestimated or if the CLT stock solution used to charge a raceway was not uniformly mixed throughout the water column, then "low" median charging doses could have resulted. If water inflow was underestimated, the calibrated water delivery system (chicken waterer plus glass siphon) may not have been adding enough CLT stock solution to the raceway during the flow-through portion of the treatment. If the delivery system was constantly "behind" in adding CLT to the raceway, then it is likely that the median CLT dose delivered would decrease through time. If the calibration system was inaccurate or inconsistent, then not enough CLT would have been delivered. However, the volume of CLT stock solution left over in the chicken waterer indicated that the proper amount of solution was delivered over the 60-min dosing period.

A calibrated chicken waterer and glass siphon are commonly used at fish hatcheries and commercial production facilities to deliver waterborne chemicals to rearing tanks or egg incubation systems. The chicken waterer functions by using a relatively large, inner holding reservoir to establish and maintain a relatively constant volume and head pressure of water (or other solution) in an outer, dispensing reservoir. Although the volume of solution in the outer reservoir remains generally constant as the volume of the inner reservoir decreases, the volume in the outer reservoir likely decreases as the volume of solution in the inner reservoir approaches near empty. Transferring 120–130% of the target volume of the chemical stock solution into the chicken waterer may have minimized the potential for decreasing the volume in the outer reservoir by avoiding decreasing the amount of solution in the inner reservoir to near-empty. Peristaltic pumps and mechanical sprayers are usually more accurate delivery systems than chicken waterers; however, such pumps and sprayers can be expensive and are also at risk of

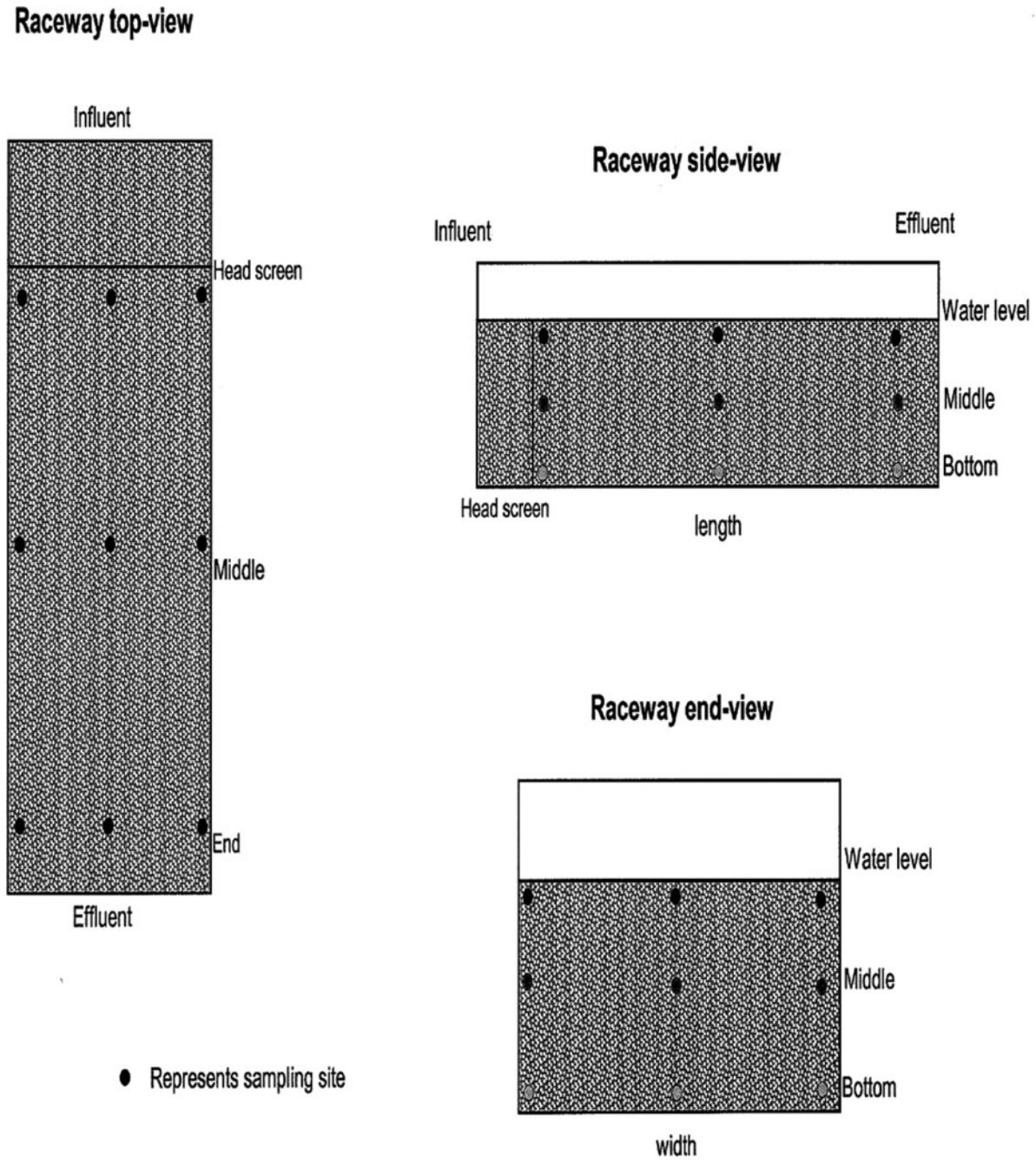

FIGURE 3. Schematic illustrating in-raceway sampling locations for CLT dose verification.

inconsistent delivery as they approach near-empty. Thus, "overfilling" might be necessary regardless of the delivery system used.

Concerns have arisen about how a concrete, rectangular culture unit may affect the flow pattern of a chemical administered as a flow-through bath, especially in such raceways that are connected in series (Rach and Ramsay 2000). Results from our study support, in whole or in part, results from studies in which therapeutic concentrations of hydrogen peroxide were verified during the course of a treatment. Saez and Bowser (2001) showed that the concentration of hydrogen peroxide could be established and maintained within ± 25% of the target dosage for 1 h only when a 750-L circular tank was pretreated to a hydrogen peroxide concentration of 100 mg/L before initiating a flow-through treatment. Rach et al. (1998) reported that the concentration of hydrogen peroxide used to control fungus on eggs

FIGURE 4. Apparatus constructed for collection of water samples from matrix of sampling locations established in the raceways.

was within 5% of the expected concentrations when sampled and analyzed 10 min into a 15-min treatment using miniature egg hatching jars. Rach and Ramsay (2000) dosed several concrete, rectangular raceways in series with hydrogen peroxide using a flow-through regimen and demonstrated that the target dosage $\pm 25\%$ was achieved in the first raceway in the series but not in the next two raceways in the series. They also found that during a 30-min flow-through treatment, there were areas in the raceways that were devoid of the hydrogen peroxide. Such a finding indicates that fish could avoid areas that contained an introduced chemical, assuming fish could sense the presence of the chemical. Rach and Ramsay (2000) hypothesized the potential for treating fish at concentrations less than the target dose for less than the expected duration, and stated that fish culturists should analytically verify the chemical therapeutant concentrations at different times and in different locations to confirm that the chemical was administered at the target dosage. A charged, flow-through treatment regimen exposed fish to the target dosage for the full duration of the treatment period, and thus there is a greater likelihood of delivering an efficacious treatment.

In our study, water samples were collected to allow us to describe dose according to a three-dimensional matrix and to evaluate treatment concentrations along a variety of strata. We presumed that concentration differences along the length of the raceway would be considered the most biologically important because preliminary testing showed that while there was little variation in water temperature and dissolved oxygen concentration (used as surrogate measures of water homogeneity) throughout the raceway, most of the variation that existed occurred along the length of the unit, not across its width or at different depths. The largest difference in overall mean CLT concentration was observed at time 0 min when the overall mean CLT concentration at the head of the raceway was 12.6 mg/L, as compared with 11.3 and 11.2 mg/L at the middle and tail end, respectively. In spite of the observed difference, means were always within 9–15 mg/L and differences were not considered biologically important. It is interesting to note that differences along the length of the raceway were probably an artifact of inaccurately charging the raceway due to the differences in water volume between the upper and lower third of the raceway, and not actually related to the flow-through portion of the treatment. However, at 30 and 60 min this pattern was reversed, with higher mean CLT concentrations present at the tail end of the raceway as compared with the head end and middle of the raceway. These differences were most likely attributed to the dynamics

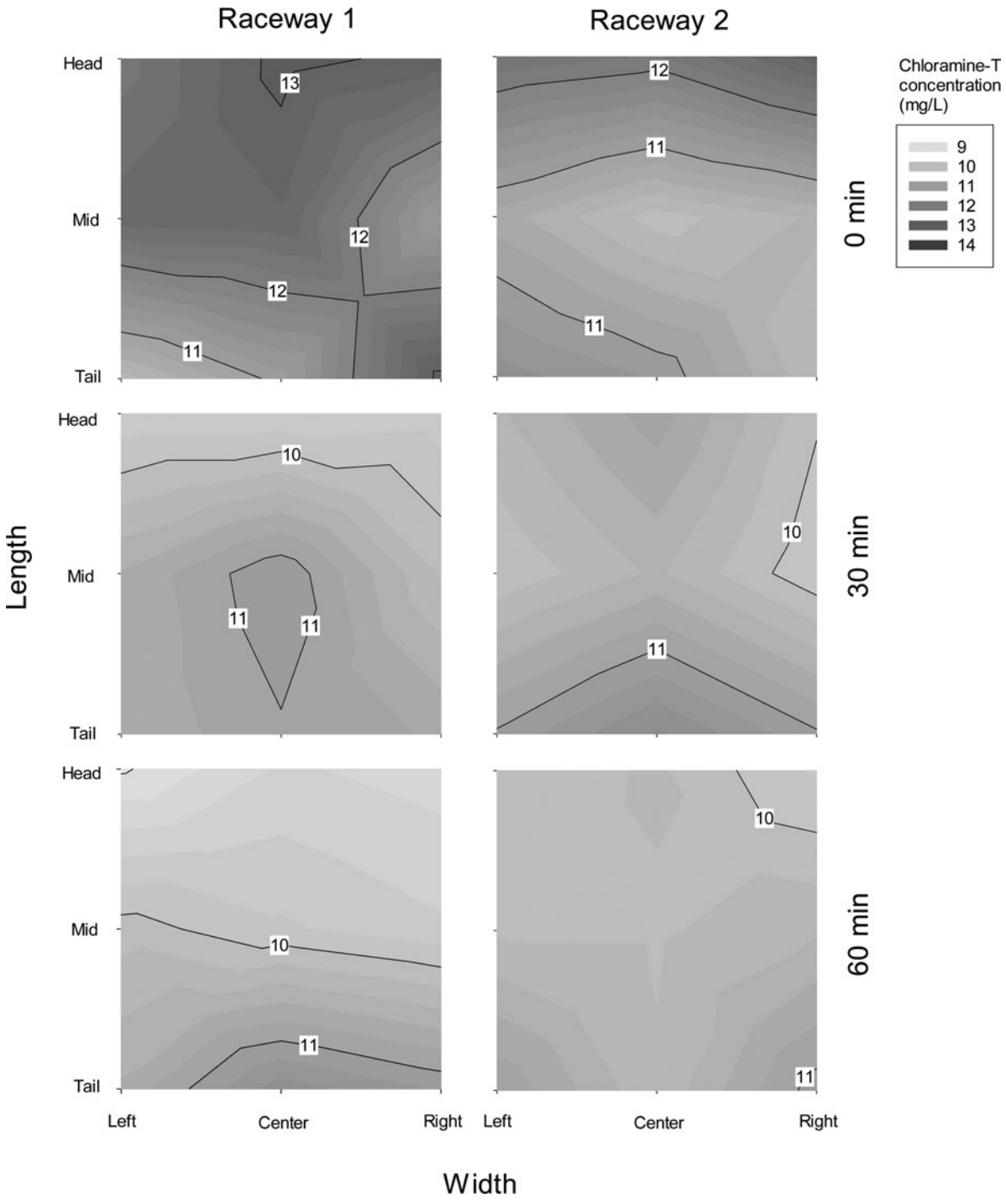

FIGURE 5. Linearly interpolated mean CLT concentration (mg/L) averaged across two independent trials and three depths plotted across space and time for both raceways.

of the flow-through portion of the treatment. It was suspected that the combined effects of variability of the CLT stock solution delivery system and difficulty of accurately measuring the flow of such a large volume of water entering the raceway may have resulted in lower-than-expected CLT concentrations in the receiving volume of water.

It is important to note that this study was conducted without fish in the raceway, thereby establishing what we considered a worst-case scenario for the uniform mixing of CLT throughout the duration of the treatment period. Obviously, fish movement throughout the water column would have facilitated a more uniform distribution of CLT. Although results from this study

indicated both acceptable accuracy and uniform distribution of CLT using a charged flow-through treatment method, it can only be assumed that a "real-world" scenario would have resulted in even more uniform results.

Based on our results, adequate data were generated to demonstrate that the acceptable target range of a waterborne chemical, such as CLT, can be achieved and maintained using a charged, flow-through treatment protocol. Thus, charged, flow-through treatments are likely to be as effective as static bath treatments from the standpoint of immersing fish at the target dose for the duration of the treatment period. However, to ensure that the target therapeutic dose is achieved and maintained, the volume of water and water inflow into a fish rearing system must be measured accurately. Measuring water volume may require measuring depth at different locations along the length of large raceways, and measuring water inflow may be challenging when dealing with high water flows. Additionally, the system used to meter a waterborne drug or chemical into a fish rearing system must be accurately calibrated, and the calibration needs to be routinely checked. Lastly, if possible, water samples should be collected periodically for dose verification purposes to ensure that the drug or chemical is being delivered at the prescribed dose.

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